44. A putative protective antigen against a Mycoplasma, prepared by a method including

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providing

a sample of a Mycoplasma;

an antibody probe including at least antibody against a Mycoplasma produced by a method including

providing a biological sample taken a short time after an immune animal has been challenged with a Mycoplasma or Mycoplasma extract taken from the infection site or an area of a lesion or an area close to the infection site or lesion;

isolating cells from the biological sample; culturing cells in vitro in suitable culture medium; andharvesting antibodies produced from said cells;

probing the Mycoplasma sample with the antibody probe to detect at least one antigen; and

isolating the antigen detected.

- 45. A putative protective antigen against Mycoplasma hyopneumoniae, or related infections, selected from the group of antigens having approximate molecular weights of 110-114, 90-94, 72-75, 60-64, 52-54 and 46-48 20 kilodaltons (kD), mutants, derivatives and fragments thereof.
- A putative protective antigen according to claim 45 wherein the antigen 46. in the 72-75 kD region contains the following N-terminal amino acid sequence: 25 AGXLQKNSLLEEVWYLAL
  - A putative protective antigen according to claim 46 further including one or more of the following internal amino acid sequences:

AKNFDFAPSIQGYKKIAHEL NLKPEQILQLLG LLKAEXNKXIEEINTXLDN

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48. A putative protective antigen according to claim 45 wherein the antigen in the 60-64 kD region contains the following N-terminal amino acid sequence:

## MKLAKLLKGFX(N/L)(M/V)IK, or ADP(F/I)(R/E)Y(V/A)PQG(Q/A)X(M/N)VG

49. A putative protective antigen according to claim 45 wherein the antigen in the 52-54 kD region contains the following N-terminal amino acid sequence:

AGXWAKETTKEEKS

10 50. A putative protective antigen according to claim 49 further including one or more of the following internal amino acid sequences:

## AWVTADGTVN AIVTADGTVNDNKPNQWVRKY

- 15 51. A putative protective antigen according to claim 45 wherein the antigen in the 46-48 kD region contains the following N-terminal amino acid sequence:

  AGXGQTESGSTSDSKPQAETLKHKV
- 52. A putative protective antigen according to claim 51 further including one or more of the following internal amino acid sequences:

TIYKPDKVLGKVAVEVLRVLIAKKNKASR AEQAITKLKLEGFDTQ KNSQNKIIDLSPEG

25 53. An isolated nucleic acid fragment encoding a putative protective antigen against Mycoplasma hyopneumoniae or related infections, said nucleic acid fragment including the following sequence, mutants, derivatives, recombinants and fragments thereof:

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	10	20	30	40	50	
	1234567890	1234567890	1234567890	1234567890	1234567890	
	ATGAAAAAA	TGCCACTATA	CCAGAGGAAA	GAGCAGTATA	TAAAATAATT	50
. 5	AAAATTACAT	TITCTTCATT	TGCGCCAGAA	TTTTTAAGAA	TTAGTACATT	100
<sub>μ</sub> 5	AAAAAGTAGA	ACAAAAGTTA	TTAATGTAAA	CATTAGCGCA	ATCCTTAAGA	150
	AAAAATTAAA	AGTTTTATCT	ATTTTTTA	ATCGAAATCC	AACCAGGCAT	200
	AAATCTTTGT	CAGTATTTAT	CAAGTCGGTA	TTTTTCATT	ATTTCTACTA	250
	TTATTATA	TGAATTTGCA	TTTTCCATAA	TCTAAAATTT	TACATTITIT	300
	TATAACAATT	TTAAAAATT	ACTCTTTAAT	TTATAGTATT	TTTTATTT	350
	TTAGTCTAAA	TTATAAAATT	ATCTTGAATT	TTATTTGAAT	TTATAATT	400
	TAGTACTAAA	AAATACAAAT	ATTTTTCCT .	ATTCTAAGAA	AAATTCATTT	450
•	TTTAAAAAAA	ATTGATTTTT	ATAGTATAAT	TTGTTTGTAT	AATTGAATTA	500
10	ACTTGATTTG	AAAGGGAACA	AAATGAAAAA	AATGCTTAGA	AAAAAATTCT	550
.5	TGTATTCATC	AGCTATTTAT		TTGCATCAAT	TATTGCATTT	600
	GTTGCAGCAG	GTTGTGGACA			CTGATTCTAA	650
	ACCACAAGCC	GAGACGCTAA	AACATAAAGT	AAGTAATGAT	TCTATTCGAA	700
	TAGCACTAAC	CGATCCGGAT	AATCCTCGAT	GAATTAGTGC	CCAAAAAGAT	750
	ATTATTTCTT	ATGTTGATGA	AACAGAGGCA	GCAACTTCAA	CAATTACAAA	800
•	AAACCAGGAT	GCACAAAATA	ACTGACTCAC	TCAGCAAGCT	AATITAAGCC	850
	CAGCGCCAAA	AGGATTTATT	ATTGCCCCTG	AAAATGGAAG	TGGAGTTGGA	900
	ACTGCTGTTA	ATACAATTGC	TGATAAAGGA	ATTCCGATTG	TTGCCTATGA	950
15	TCGACTAATT	ACTGGATCTG	ATAAATATGA	TTGGTATGTT	TCTTTTGATA	1000
	ATGAAAAAGT	TGGTGAATTA	CAAGGTCTTT	CACTTGCTGC	GGGTCTATTA	1050
	GGAAAAGAAG	ATGGTGCTTT	TGATTCAATT	GATCAAATGA	ATGAATATCT	1100
	AAAATCACAT	ATGCCCCAAG	AGACAATTTC	TTTTTATACA	ATCGCGGGTT	1150
	CCCAAGATGA	TAATAATTCC	CAATATTTTT	ATAATGGTGC	AATGAAAGTA	1200
	CTTAAAGAAT	TAATGAAAAA	TTCGCAAAAT	AAAATAATTG	ATTTATCTCC	1250
	TGAAGGCGAA	AATGCTGTTT	ATGTCCCAGG	ATGAAATTAT		1300
	GTCAAAGAAT	CCAATCTTTT	CTAACAATTA	ACAAAGATCC		1350
	AATAAAATCA	AAGCTGTTGG	TTCAAAACCA	GCTTCTATTT	TCAAAGGATT	1400
20	TCTTGCCCCA	AATGATGGAA	TGGCCGAACA	AGCAATCACC	AAATTAAAAC	1450
	TTGAAGGGTT	TGATACCCAA	AAAATCTTTG	TAACTCGTCA	AGATTATAAT	1500
	GATAAAGCCA	AAACTTTTAT	CAAAGACGGC	GATCAAAATA	TGACAATITA	1550
	TAAACCTGAT	AAAGTTTTAG	GAAAAGTTGC	TGTTGAAGTT	CTTCGGGTTT	1600
	TAATTGCAAA	GAAAAATAAA	GCATCTAGAT	CAGAAGTCGA	AAACGAACTA	1650
	AAAGCAAAAC	TACCAAATAT	TTCATTTAAA	TATGATAATC	AAACATATAA	1700
	AGTACAAGGT	ATATAAAA	ATACAATTTT	AGTAAGTCCA	GTAATTGTTA	1750
	CAAAAGCTAA	TGTTGATAAT	CCTGATGCCT	AA		1782
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54. A method for producing an antibody against a <u>Mycoplasma</u> including providing a biological sample taken a short time after an immune animal has been challenged with a <u>Mycoplasma</u> or <u>Mycoplasma</u> extract taken from the infection site or an area of a lesion or an area close to the infection site or lesion;

isolating cells from the biological sample;

culturing cells in vitro in a suitable culture medium; and harvesting antibodies produced from said cells.

CONTIN

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- 55. A method according to claim 54 wherein the biological sample is taken approximately 2 to 7 days after the animal has been challenged with the Mycoplasma.
- 56. A method according to claim 55 wherein the culturing of cells in vitro further includes addition of helper factors to the culture, said helper factors selected from the group including cytokines used alone or in combination, including interleukin 1, 2, 3, 4, 5, 6, 7 and 8, colony stimulating factors, interferons and any other factors that may be shown to have an enhancing effect on specific B cell secretion.
- 15 57. A method according to claim 56 further including a cell activation step including activating the cells isolated to proliferate and secrete and/or release antibodies, said cell activation step including adding a cell activating agent to the culture medium, said cell activating agent selected from the group including mitogens.

  / and helper factors produced by leukocytes, or their synthetic equivalents or combinations thereof.
  - 58. A method according to claim 57 wherein the antibody is in the form of the supernatant harvested from the culture medium.
- 25 59. An antibody against a <u>Mycoplasma</u> prepared according to the method of claim 54.
  - 60. A method of identifying a putative protective antigen associated with a <a href="Mycoplasma"><u>Mycoplasma</u></a>, said method including
- 30 providing

a sample of a Mycoplasma; and an antibody probe including at least one antibody against a

## Mycoplasma;

probing the <u>Mvcoplasma</u> sample with the antibody probe to detect at least one antigen; and

isolating the antigen detected.

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61. A method of purifying a putative protective antigen associated with a <a href="Mycoplasma">Mycoplasma</a>, said method including providing

a crude antigen mixture; and

an antibody against a Mycoplasma immobilized on a suitable

10 support;

subjecting the crude antigen mixture to affinity chromatography utilizing the immobilized antibody; and

isolating the purified antigen so formed.

15 62. A method for preparing a synthetic antigenic polypeptide against Mycoplasma, which method includes

providing

a cDNA library or genomic library derived from a sample of Mycoplasma, and

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an antibody probe including an antibody prepared according to claim 54:

generating synthetic polypeptides from the cDNA library or genomic library;

probing the synthetic polypeptides with the antibody probe; and isolating the synthetic antigenic polypeptide detected thereby.

63. A method according to claim 62 wherein the antibody probe includes an antibody raised against an antigen against <u>Mycoplasma hyopneumoniae</u>, or related infections selected from the group of antigens having approximate molecular weights of 110-114, 90-94, 72-75, 60-64, 52-54 and 46-48 kilodaltons (kD).

mutants, derivatives and fragments

thereof.

CONTIP

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64. A synthetic putative protective antigen produced by the method of claim 62.

- 65. A vaccine or veterinary composition including a prophylactically effective amount of at least one putative protective antigen against a <u>Mycoplasma</u> according to claim 45.
  - 66. A vaccine or veterinary composition according to claim 65 including a plurality of putative protective antigens.
  - 67. A vaccine or veterinary composition including an antibody against a Mycoplasma according to claim 59.
  - 68. A diagnostic kit including an antigen according to claim 45.
  - 69. A method for preventing or treating a <u>Mycoplasma</u> infection, which method including administering to an animal a prophylactically or therapeutically effective amount of at least one putative protective antigen according to claim 45.
- 70. An isolated DNA fragment encoding a putative protective antigen against <u>Mycoplasma</u> or related infections, said DNA fragment having a nucleic acid sequence according to Figure 6 or an homologous sequence, and functionally active fragments, mutants, variants or recombinants thereof.
- 71. A clone including a DNA fragment according to claim 70.
- 72. A clone according to claim 71 which is clone pC1-2
- 73. An amino acid sequence or functional equivalent thereof encoded by the DNA fragment according to claim 70.

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